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Current Literature
In Basic Science

Chloride's Exciting Role in Neonatal Seizures Suggests Novel Therapeutic Approach

Progressive NKCC1-Dependent Neuronal Chloride Accumulation During Neonatal Seizures.

Dzhala VI, Kuchibhotla KV, Glykys JC, Kahle KT, Swiercz WB, Feng G, Kuner T, Augustine GJ, Bacskai BJ, Staley KJ. *J Neurosci* 2010;30(35):11745–11761.

Seizures induce excitatory shifts in the reversal potential for $GABA_A$-receptor-mediated responses, which may contribute to the intractability of electro-encephalographic seizures and preclude the efficacy of widely used GABAergic anticonvulsants such as phenobarbital. We now report that, in intact hippocampi prepared from neonatal rats and transgenic mice expressing Clomeleon, recurrent seizures progressively increase the intracellular chloride concentration ($[Cl^-]i$) assayed by Clomeleon imaging and invert the net effect of $GABA_A$ receptor activation from inhibition to excitation assayed by the frequency of action potentials and intracellular Ca^{2+} transients. These changes correlate with increasing frequency of seizure-like events and reduction in phenobarbital efficacy. The Na^+-K^+-$2Cl^-$ (NKCC1) cotransporter blocker bumetanide inhibited seizure-induced neuronal Cl^- accumulation and the consequent facilitation of recurrent seizures. Our results demonstrate a novel mechanism by which seizure activity leads to $[Cl^-]i$ accumulation, thereby increasing the probability of subsequent seizures. This provides a potential mechanism for the early crescendo phase of neonatal seizures.

Commentary

Inhibition in the central nervous system is dependent on chloride conductance through $GABA_A$ receptor-associated channels. When chloride channels open, the membrane potential is driven toward chloride's reversal potential, and because chloride's reversal potential is negative to the neuron's threshold for firing action potentials, it normally has an inhibitory effect. $GABA_A$ receptors have binding sites for both barbiturates and benzodiazepines, allowing them to enhance the inhibitory effect of GABA at this receptor; it is this mechanism of action that is responsible for the anticonvulsant activity of these agents.

Prolonged seizure activity reduces the efficacy of GABAergic antiepileptic drugs (AEDs) such as benzodiazepines and barbiturates. If postsynaptic $GABA_A$ receptor responsiveness was reduced, one would expect GABAergic agents to lose efficacy as well. Indeed, a gradual suppression of $GABA_A$ receptor responses occurs during in vitro kindling (1) and GABA receptor internalization during ongoing seizures and status epilepticus has been documented (2), accounting for pharmacoresistance to GABAergic agents in these settings.

One might also expect reduced inhibitory efficacy to result from intracellular buildup of chloride: prolonged neuronal activity results in elevated extracellular potassium concentrations, and this potassium is cleared in part by neuronal transporters that bring potassium back into the cells. NKCC1 is one such transporter, and when this transporter brings potassium back into the cells, it imports chloride with it. The resultant elevation of intracellular chloride will reduce efficacy of $GABA_A$ receptor-mediated synaptic inhibition by making the chloride reversal potential less negative. However, in the mature nervous system, the KCC2 transporter will do its best to counteract this chloride buildup by extruding chloride and restoring normal homeostasis.

In the immature nervous system, however, the KCC2 transporter is not fully expressed. This makes the neonate more vulnerable to intracellular chloride buildup mediated by the NKCC1 transporter. Indeed, immature neurons have elevated baseline concentrations of intracellular chloride; as a result, neonatal $GABA_A$ receptor responses are not only less inhibitory, they may actually be excitatory (3, 4). The excitatory action of GABA-mediated chloride conductance is believed to contribute to the increased incidence of seizures in human neonates (5). There is a progressive developmental increase in expression of the chloride-extruding transporter KCC2 in the brain that eventually allows for the full expression of hyperpolarizing $GABA_A$ receptor responses; in the rat hippocampus, this occurs by the end of the second postnatal week (6).

Numerous experiments have been done examining the efficacy of barbiturates in neonatal seizure models in vitro and in vivo, with conflicting results (7, 8). The current paper by Dzhala and colleagues attempts to make sense of this controversial area of investigation. The authors propose that the differences in response to GABAergic AEDs may correlate with

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timing of treatment relative to seizure onset and recurrence. They test the hypothesis that seizure duration and recurrence induce a progressive NKCC1-driven change in chloride reversal potential that underlies the reduction in inhibitory efficacy and seizure exacerbation over time. They therefore suggest that blocking the NKCC1 transporter may be an effective approach to reducing chloride accumulation, thereby suppressing $GABA_A$ receptor-mediated excitation, ameliorating the continued seizure activity, and restoring the efficacy of GABA-modulating AEDs.

The investigators performed extracellular electrophysiologic recordings from intact isolated hippocampi from neonatal rats. They also used transgenic CLM-1 mice expressing Clomeleon, which allowed for high-resolution two-photon chloride imaging of CA3 pyramidal cells and interneurons. Although their findings were at times contradictory, their conclusions seem valid and potentially important.

They first exposed the hippocampi to a $GABA_A$ receptor agonist and demonstrated an inhibitory effect, as evidenced by an overall suppression of spontaneous neuronal firing. This was somewhat surprising in light of numerous previous slice studies in which excitatory GABA responses have been demonstrated at this developmental stage. The authors suggest, based on their measurements of intracellular chloride concentration, that neurons at this stages are heterogeneous in this regard, with some having sufficiently elevated levels of chloride to produce excitatory GABA responses but most expressing inhibition.

The authors proceeded to monitor chloride levels in CA3 pyramidal cells expressing recurrent seizure discharges and demonstrated progressive increases in intracellular chloride concentration with recurrent seizures; this increase persisted for hours following termination of the final seizure, suggesting lowered seizure threshold persists during this time as a result of deficient GABA inhibition. They then showed that the reduction of barbiturate efficacy was also proportional to the number of preceding seizures, correlating with the increase in intracellular chloride.

It has previously been shown that prolonged seizure activity (status epilepticus) causes excitatory shifts in GABA reversal potential, reducing inhibitory efficacy of GABA (9). What this paper adds to the story is that this effect is progressive with recurrent seizures: the more seizures, the more intracellular chloride rises; and the more chloride rises, the more excitatory the $GABA_A$-mediated response. Furthermore, this effect can persist for hours following recurrent seizures, reducing seizure threshold during that time as well.

The most impressive data in this paper are shown in Figure 9: after first demonstrating a baseline net inhibitory action of GABA agonist, GABA responses are elicited following an episode of status epilepticus, and the GABA agonist at this time point elicits an excitatory response and continues to do so for hours following seizure termination. When the experiment is performed in the presence of bumetanide, an NKCC1 antagonist, the baseline inhibitory effect of the GABA agonist is retained following status epilepticus, convincingly demonstrating an NKCC1-dependent mechanism for the seizure-induced enhanced hippocampal excitability mediated by reversal of GABA effect from inhibitory to excitatory.

Bumetanide is an FDA-approved diuretic that is available on the market. Its inhibitory effect at the NKCC1 transporter suggests possible additional utility in suppressing neonatal seizures, especially ongoing status epilepticus or recurrent clusters of seizures that have become refractory to GABA-modulating AED therapies. Regrettably, studies with other seizure models do not consistently find this agent to be efficacious in suppressing neonatal seizures (10, 11). And although bumetanide may help suppress acute prolonged seizures, such treatment has not been effective in preventing kindling epileptogenesis of secondary seizure foci (11). Nevertheless, the results of Dzhala et al. do suggest that barbiturate resistance may indeed be ameliorated by concurrent administration of bumetanide. Although many questions remain to be answered, the authors are to be commended for making a valiant effort to resolve the controversies regarding varying barbiturate efficacy in neonatal seizures and for suggesting a potential novel adjunctive therapy utilizing a clear mechanistic approach. Indeed, a better understanding of seizure mechanisms in different epilepsy syndromes provides the opportunity to craft improved therapeutic regimens.

by Lisa R. Merlin, MD

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